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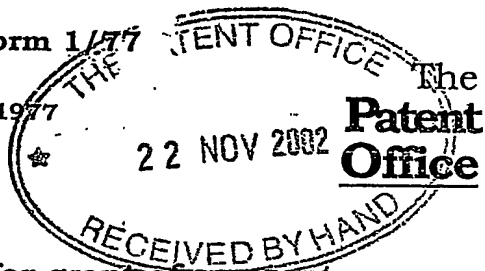
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Dated 16 September 2003



1/77

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2.	Patent application number (The Patent Office will fill in this part)	0227352.2		22 NOV 2002
3.	Full name, address and postcode of the or of each applicant (underline all surnames)	NOVARTIS AG LICHTSTRASSE 35 4056 BASEL SWITZERLAND Patent ADP number (if you know it) 7125487005 If the applicant is a corporate body, give the country/state of its incorporation SWITZERLAND		
4.	Title of invention	Organic compounds		
5.	Name of your agent (If you have one)  "Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	B.A. YORKE & CO. CHARTERED PATENT AGENTS COOMB HOUSE, 7 ST. JOHN'S ROAD ISLEWORTH MIDDLESEX TW7 6NH Patents ADP number (if you know it) 1800001		
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B. A. Yorke & Co

B.A. Yorke & Co.

22 November 2002

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## Organic Compounds

### **FIELD OF THE INVENTION**

The invention is in the field of nucleic acid chemistry and concerns methods for the preparation and use of ribonucleoside derivatives with novel protecting groups. The inventive compounds are particularly adapted for the automated preparation of oligoribonucleotides.

### **BACKGROUND OF THE INVENTION**

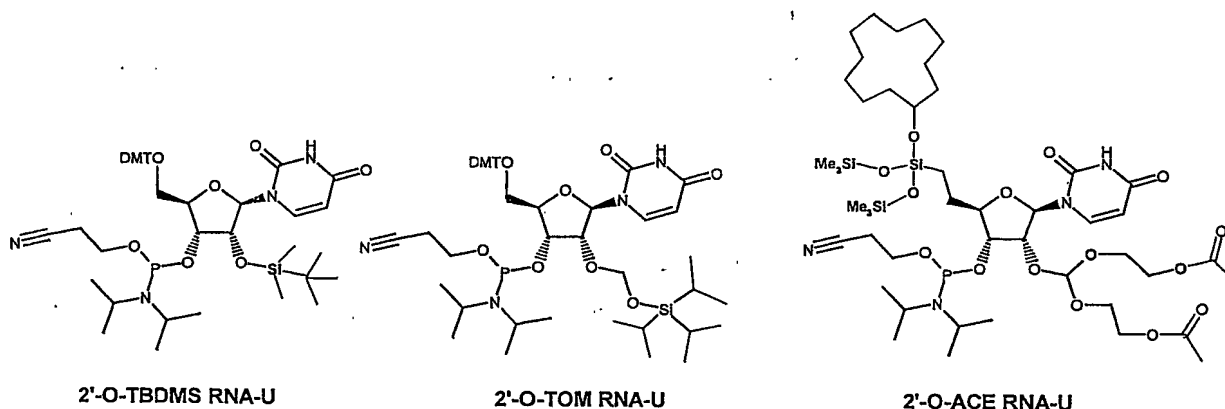
Applications for synthetic nucleic acids are numerous and key for the understanding of biological processes. Among these applications, the use of synthetic oligonucleotides for the specific down-regulation of proteins by specific hybridisation in the cell of the synthetic oligonucleotide to a mRNA is known as an antisense mechanism and has been widely described (1). More recently, RNA interference, a technique using dsRNA known as siRNA, has been successfully used to inhibit the translation of mammalian mRNA to its protein (2). The great promise of RNA technology has created a need for the development of efficient and cost effective preparation of synthetic oligoribonucleotides.

Oligoribonucleotide synthesis is more challenging than oligodeoxynucleotide synthesis, mainly because of the 2'-OH group which is present in ribonucleic acids, but not in deoxyribonucleic acids. Chemical synthesis of oligoribonucleotides is normally based on a protected ribonucleoside derivative immobilized on a solid phase to which further protected ribonucleotide derivatives are coupled in consecutive steps of one synthesis cycle each until the desired length of chain is achieved. To ensure an efficient synthesis and to avoid RNA degradation during the preparation process, the protecting group strategy for the 2'-OH group should be perfectly orthogonal with that of other protecting groups and the protecting group should be removed as late as possible in the process. So far, mainly the following types of protection groups have been used to protect the 2'-OH group:

2'-O-TBDMS chemistry is the commonly used protecting group for RNA synthesis (3). It is orthogonal with other protecting groups. However, 2'-3' phosphoryl migration during oligoribonucleotide synthesis has been reported (4). Moreover, steric hindrance of t-butyl(dimethylsilyl) group close to reactive phosphoramidite significantly diminishes coupling efficiency. The latter limitation can be reduced but at the price of longer coupling times, the use of higher molar excesses of reagents and special phosphoramidite activators like 5-(Benzylmercapto)-1H-tetrazole for instance (5).

2'-O-ACE chemistry has been described by Caruthers et al. (6) as an alternative to TBDMS chemistry. There, 2'-OH is protected by an acid labile orthoester. As compared to TBDMS, lower hindrance of that protecting group allows higher coupling rates. The acid lability of 2' orthoester requires a non-acid labile temporary protection of the 5'-OH. This was accomplished with trisubstituted silyl groups which are removed at the end of each coupling cycle by a fluoride-containing solution. Consequently, reagents for the preparation of 2'-O-ACE building blocks have to be scaled up specifically. Such 5' deprotection may in some cases be problematic: it requires a dedicated synthesizer resistant to fluoride ions and the use of the commonly used silica based supports (like Controlled Pore Glass) is not possible. 2'-O-TOM chemistry has been reported by Pitsch et al. (6). As for 2'-OTBDMS protecting group strategy, 2'-O-TOM protecting group is removed upon treatment with fluoride ions. Originally, it was developed to allow the synthesis of long RNA without the 2'-3' phosphoryl migration observed with 2'-O-TBDMS. In this case, due to the acetal nature of the bond between nucleoside and protection-group, no migration of the protection-group to a different position in the ribonucleoside-derivative, in particular to the neighbouring 3'-O-position, can occur. Such isomerization is a well known problem in the synthesis of the conventional 2'-O-silyl-substituted RNA-units.

An additional important advantage of this protecting group is the lower hindrance of the protecting group due to the acetal spacer between 2'-oxygen and the bulky triisopropylsilyl group.



Both 2'-O-TOM and 2'-O-ACE are affording coupling yields approaching those observed in oligodeoxynucleotide synthesis. Satisfying coupling yields are also obtainable with 2'-O-TBDMS chemistry but at price of use of unusual activators or of higher molar excesses of building blocks. In all cases, the building blocks mentioned are contributing to a large extent to the manufacturing costs of oligoribonucleotides. Secondly, post-synthetic processing of

oligoribonucleotides is more demanding as compared to processing of oligodeoxyribonucleotides. The latter aspect can be of special importance when high-throughput demand has to be satisfied.

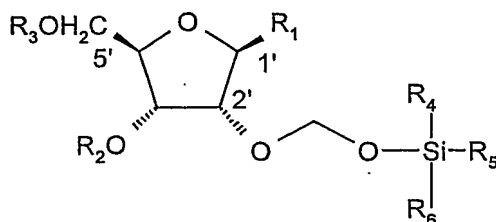
There is a need for improved RNA building blocks which are easily affordable and allowing an easier post-synthetic processing.

Substituted silyloxymethyl groups have been used as protecting groups of hydroxyl groups in the past (7,8). Steric hindrance of substituents on Si atom modulates stability and removal conditions of the protecting group. For instance, silyloxymethyl bearing tertiary carbons vicinal to the Si atom have been reported (7), in these cases, yields for removal of protecting groups were suboptimal as compared to those usually observed with TBDMS protection, and apparently not suited for solid phase oligoribonucleotide synthesis.

The present invention now provides novel and improved groups for the protection at the 2'-OH position of ribonucleosides derivatives that are particularly suited for automated oligoribonucleotide solid phase synthesis. A new procedure for the preparation of these building blocks is provided. Finally, the use of these building blocks in RNA solid phase synthesis is disclosed.

#### SUMMARY OF THE INVENTION

In one aspect, the present invention provides ribonucleoside-derivatives of the formula



wherein

$R_1$  is a base of the purine- or pyrimidine-family or a derivative of such a base or any other residue which serves as a nucleobase surrogate,

$R_2$  is a proton or a substituted derivative of phosphonic acid,

$R_3$  is a proton or a protection-group for the oxygen atom in 5'-position,

$R_4$ ,  $R_5$  and  $R_6$  are alkyl or aryl or a combination of alkyl and aryl or heteroatom,  $R_4$ ,  $R_5$  and  $R_6$  may also be cyclically connected to each other;

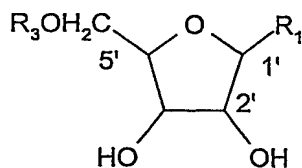
and

wherein at least one of the  $R_4$ ,  $R_5$  or  $R_6$  substituents comprises a tertiary C-atom or a heteroatom vicinal to the Si-atom.

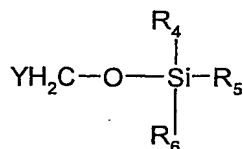
In a preferred aspect, the substituent comprising the tertiary C-atom vicinal to the Si-atom comprises from 4 to 24 C-atoms, more preferably from 5 to 24 C-atoms and yet more preferably from 6 to 24 C-atoms. In a more preferred aspect, the substituent comprising the tertiary C-atom vicinal to the Si-atom is an alkyl-substituent selected from the group consisting of tert-butyl, tert-pentyl, tert-hexyl, tert-heptyl, tert-octyl, tert-nonyl, tert-decyl, tert-undecyl, tert-dodecyl. In a another preferred aspect, the substituent comprising the tertiary C-atom vicinal to the Si-atom is selected from the group of 1,1-dimethyl ethyl, 1,1-dimethyl-propyl, 1,1-dimethyl-butyl, 1,1-dimethyl-pentyl, 1,1-dimethyl-hexyl, thexyl (1,1,2-trimethyl-propyl), 1,1,2-trimethyl-butyl, 1,1,2-trimethyl-pentyl, 1,1,2-trimethyl-hexyl, 1,1,2,2 tetramethyl-propyl, 1,1,2,2-tetramethyl-butyl. In a more preferred embodiment, the substituents of the above groups comprises at least 5 C-atoms, more preferably at least 6 C-atoms.

In a related aspect, the present invention provides ribonucleoside-derivatives wherein the substituent vicinal to the Si-atom comprises a substituted heteroatom. In a preferred aspect, the substituent vicinal to the Si-atom comprises a substituted bivalent heteroatom, in a more preferred aspect this substituent is oxygen.

In another aspect, the present invention provides a method for the preparation of a ribonucleoside-derivatives, comprising reacting a nucleoside with the formula

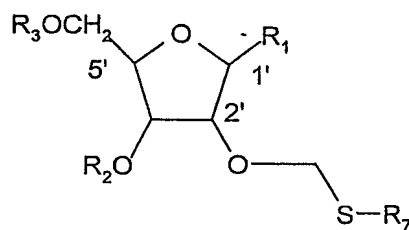


where  $R_1$  and  $R_3$  are as defined as above, with a silyloxymethyl derivative of the formula

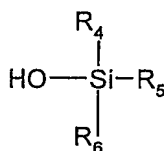


wherein Y is a suitable leaving group and wherein R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> are alkyl or aryl or a combination of alkyl and aryl or a heteroatom, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> may also be cyclically connected to each other. In a preferred embodiment Y is halogen. In another preferred embodiment, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> together comprise between 6 and 30 carbon atoms. In a further preferred embodiment, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> comprise at least one substituted heteroatom vicinal to Si atom, which is preferably a bivalent atom, more preferably oxygen. The ribonucleoside derivative may further be substituted on the oxygen in 3'-position with a group comprising of a derivative of phosphonic acid.

Another aspect of the present invention provides a method for the preparation of a ribonucleoside-derivative, comprising reacting a ribonucleoside derivative with the formula



upon an electrophilic activation with a compound of formula:



wherein R<sub>1</sub> is defined as above and R<sub>7</sub> is a alkyl- or aryl-group, or alkyl-aryl-group,  
 wherein R<sub>2</sub> is a protecting group,  
 wherein R<sub>3</sub> is a protecting group,  
 wherein R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> are defined as above.

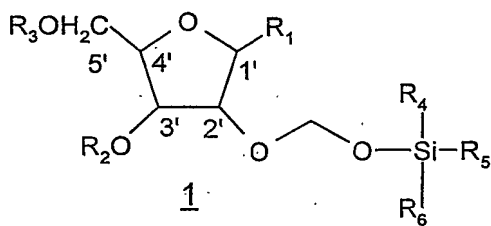
The method of claim 15 or 16 wherein the ribonucleoside derivative is further substituted on the oxygen in 3'-position with a group comprising of a derivative of phosphonic acid.



**DETAILED DESCRIPTION OF THE INVENTION**Abbreviations:

TBDMS	t-butyl dimethylsilyl
ACE	bis[2-(acetyloxy)ethoxy]methyl
TOM	(triisopropylsilyl)oxymethyl
THEX	[[(1,1,2-trimethyl-propyl)-dimethylsilyl]]-oxymethyl
DCA	Dichloroacetic acid
dsRNA	Double-stranded RNA
siRNA	Small interfering RNA

The present invention relates to 2'-O-silyloxymethyl ribonucleotide-derivatives for application in the chemical synthesis of ribonucleic acids comprising a D or L-ribose unit having the following general structural formula:



whereby

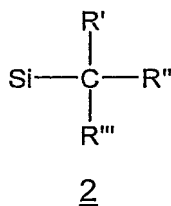
$R_1$  is a base of the purine- or pyrimidine-family or a derivative of such a base or any other residue which serves as a nucleobase surrogate,  $R_2$  is a proton or a substituted derivative of phosphonic acid,  $R_3$  is a proton or a protection-group for the oxygen atom in 5'-position, and  $R_4$ ,  $R_5$  and  $R_6$  are alkyl- or aryl-groups or a alkyl-aryl-group.  $R_4$ ,  $R_5$  and  $R_6$  may also be cyclically connected to each other.

The protection group  $R_3$  in 5'-O-position is e.g. a monomethoxytrityl- or dimethoxytrityl-group or a different, suitable group which is removed from the growing sequence during chain building such freeing a bonding position for coupling the next unit to be added to the chain.

The base component  $R_1$  of the ribonucleoside derivative is preferably a base of the purine or pyrimidine family, e.g. one of the five nucleobase adenine, cytosine, thymine, uracil, guanine or a derivative thereof, or any other residue which serves as a nucleobase surrogate. It can be protected by an acyl-substituent which can be removed after chain creation.

In the 3'-O-position,  $R_2$  is a derivative of phosphonic acid, such as an N,N- and O-substituted phosphoramidite group, whereby the N-substituents are alkyl- or aryl-groups which can be further substituted and /or cyclically connected to each other. By activation of the nitrogen of the disubstituted amino-group the phosphorus centre is activated for coupling the unit to a growing chain.

This invention now provides new and advantageous 2'-O-silyloxymethyl protecting groups wherein  $R_4$ ,  $R_5$  or  $R_6$ , is an alkyl- or aryl-substituent, or an alkyl-aryl- or aryl-alkyl or a substituted heteroatom substituent, and wherein at least one of the  $R_4$ ,  $R_5$  or  $R_6$  substituents comprises a heteroatom or a tertiary C-atom as can be represented by the formula



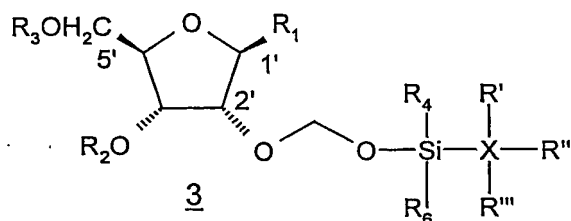
wherein  $R'$ ,  $R''$  and  $R'''$  are alkyl- or aryl, or an alkyl-aryl-substituent- or aryl-alkyl or a substituted heteroatom, and wherein  $R'$ ,  $R''$  and  $R'''$  are not H.  $R'$ ,  $R''$  and  $R'''$  may be the same or different, preferred are substituents comprising 1 to 12 C-atoms, preferably 1 to 6 C-atoms and more preferably are 1 to 4 C-atoms.  $R'$ ,  $R''$  and  $R'''$  may also be cyclically connected to each other, for instance  $R'$  may be cyclically connected to  $R''$  or  $R'''$ , or  $R''$  may be cyclically connected to  $R'''$ . In a preferred embodiment, two of the substituents are identical and comprise from 1 to 6 C-atoms, preferably from 1 to 4 C-atoms. The third substituent comprises preferably at least 3 C-atoms, preferred are from 3 to 12 C-atoms, more preferred are from 3 to 6 C-atoms.

Thus, in one embodiment at least one of the substituents  $R_4$ ,  $R_5$  and/or  $R_6$  is ( $C_4$  to  $C_{24}$ )-tertiary-alkyl and/or aryl, preferably ( $C_5$  to  $C_{18}$ )-tertiary-alkyl and/or aryl, more preferably ( $C_6$

to C<sub>12</sub>)-tertiary-alkyl and/or aryl, wherein the tertiary C-atom is vicinal to the Si-atom. Without intending to be limited to these groups, examples of such substituents may comprise for instance tert-butyl, tert-pentyl, tert-hexyl, tert-heptyl, tert-octyl, tert-nonyl, tert-decyl, tert-undecyl, tert-dodecyl, hexyl (1,1,2-trimethyl-propyl), 1,1,2-trimethyl-butyl, 1,1,2-trimethyl-pentyl, 1,1,2-trimethyl-hexyl, 1,1,2,2 tetramethyl-propyl, 1,1,2,2-tetramethyl-butyl. In a preferred embodiment, the substituent is tert-pentyl or higher, in a more preferred embodiment the substituent is tert-hexyl or higher. More preferred examples comprise e.g. 1,1 dimethyl-ethyl, 1,1-dimethyl-propyl, 1,1-dimethyl-butyl, 1,1-dimethyl-pentyl, 1,1-dimethyl-hexyl, 1,1,2-trimethyl-propyl, 1,1,2-trimethyl-butyl, 1,1,2-trimethyl-pentyl, 1,1,2-trimethyl-hexyl, 1,1,2,2 tetramethyl-propyl, 1,1,2,2-tetramethyl-butyl. In another embodiment R<sub>4</sub>, R<sub>5</sub> and/or R<sub>6</sub> comprise a heteroatom:

The substituent(s) which do not comprise a tertiary C-atom may have identical or different substituents. These substituents are preferably alkyl- or aryl- substituents, or alkyl-aryl-substituents. Preferred are substituent comprising from 1 to 12 C-atoms, preferably from 1 to 8 C-atoms, more preferably from 1 to 4 C-atoms. Without intending to be limited to these groups, examples of such substituents may comprise for instance methyl, ethyl, propyl, butyl, pentyl, hexyl, i-propyl, sec-butyl, isobutyl, sec-pentyl.

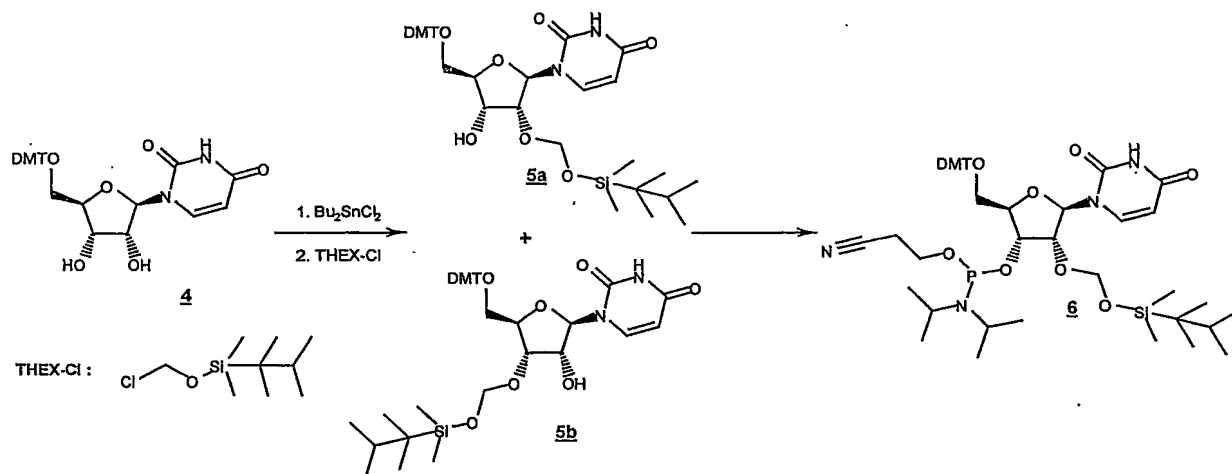
In another embodiment, R<sub>4</sub>, R<sub>5</sub> and/or R<sub>6</sub> comprise a substituted heteroatom like for instance silicon, germanium, tin, lead, nitrogen, oxygen, sulfur such as for instance can be represented for a "four-valent" heteroatom by the formula:



wherein X is Si or Ge, Sn or Pb and wherein R', R'' and R''' are defined as for formula 2, and R<sub>4</sub> and R<sub>6</sub> are defined as above. Any of the substituents R<sub>4</sub>, R<sub>5</sub> or R<sub>6</sub> may comprise the heteroatom vicinal to the Si-atom, preferred only one as is exemplified in formula 3. For "bivalent" heteroatoms like oxygen, or for trivalent heteroatoms like nitrogen formula 3 may be adapted accordingly as a person of skill in the art would readily recognize. In a preferred embodiment of the present invention, X is oxygen.

The compound may be prepared by methods known in the art such as via the organometallic route. This route is described for instance in WO99/09044 (6). The reaction 4  $\rightarrow$  5a/5b  $\rightarrow$  6 shows an example of the preparation of a compound of the present invention via the organometallic route. Briefly, a ribonucleoside protected at the 5'-O is reacted with e.g. chloromethyl[dimethyl-(1,1,2-trimethylpropyl)silyl]ether (or THEX-Cl) in the presence of a suitable organometallic salt, such as for instance dibutyltin dichloride or dibutyltin oxide. THEX-Cl itself is prepared in a manner similar to TOM-Cl according to a published procedure (7). However, unlike TOM-Cl, the preparation of THEX-Cl does not require a final distillation step of the reagent prior to the reaction with the ribonucleoside which represents a significant simplification. In the reaction of THEX-Cl with a 5'-O-protected ribonucleoside a mixture of 2'- and 3'-protected ribonucleosides is obtained, wherefrom the 2'-substituted ribonucleoside is purified by for instance chromatographic means. In a subsequent step the 3'-OH group of the purified compound 5a is converted to the phosphoramidite 6 according to methods known in the art (Sinha, N.D. et al., Tetrahedron Lett. 1983, 24, 5843; Sinha, N.D. et al., Nucleic Acid Res. 1984, 12, 4539).

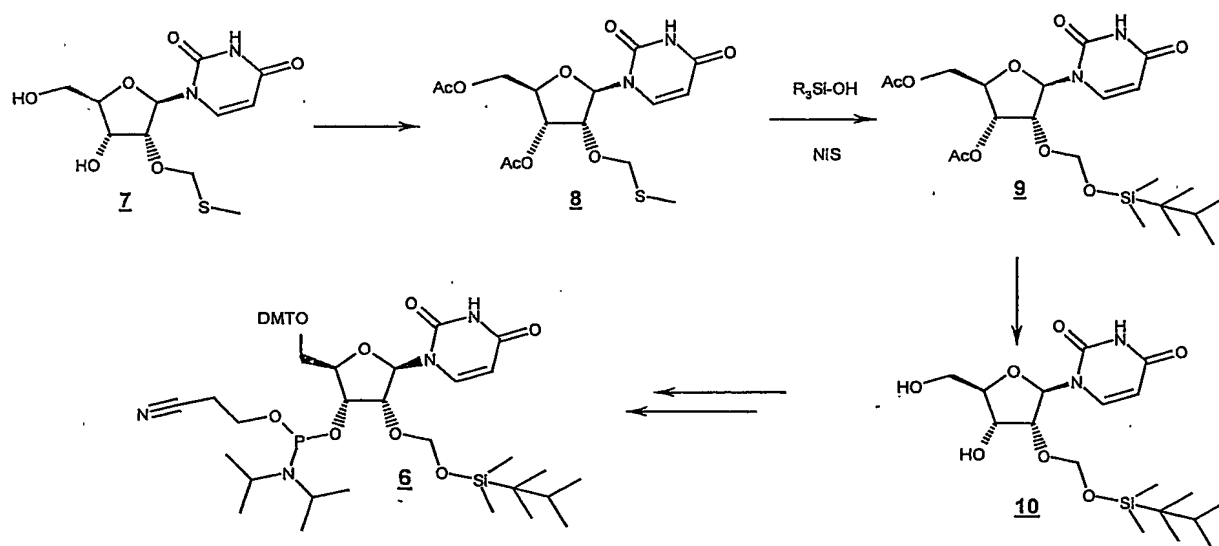
This method requires the use of substituted silyloxymethyl ethers wherein substituents contain alkyl, aryl, or arylalkyl groups and which can be cyclically connected. In a further embodiment, this method is applicable to silyloxymethylethers wherein at least one of the substituents contains at least one substituted heteroatom like for instance silicon, germanium, tin, lead, nitrogen, oxygen, sulfur



The reaction may be carried out in solution or on solid phase or by using polymer supported reagents. The solvent can be a hydrocarbon solvent, ethereal solvent, nitrile solvent, chlorinated solvent, heterocyclic solvent, sulfoxide solvents, etc.. Specific examples of suitable solvents include pyridine, N,N-dimethylformamide (DMF), tetrahydrofuran (THF), dimethylsulfoxide (DMSO), acetonitrile, dichloroethane and methylene chloride. Preferably, dichloroethane is used.

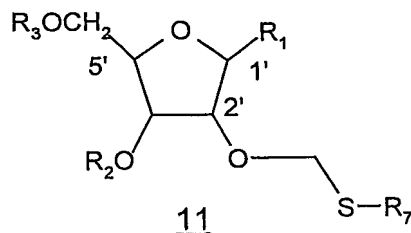
Although the reaction may be carried out at room temperature, it may also be carried out at a temperature range of 0 to 150°C preferably at 10 to 100°C.

In a further aspect of the present invention, the compounds of the invention are prepared by a new and superior method as exemplified by the reaction  $\underline{7} \rightarrow \underline{8} \rightarrow \underline{9} \rightarrow \underline{10} \rightarrow \underline{6}$ .



This method allows the selective introduction of 2'-OH protecting groups via first introducing a 2'-O-alkylthiomethyl, arylthiomethyl, alkylarylthiomethyl or arylalkylthiomethyl group. It avoids the unselective step described in the organometallic route. This new method is generally applicable for the introduction of oxymethyl derivatives selectively on 2'-OH group of ribonucleosides and is not restricted to the introduction of protecting groups as described above. The methods for the preparation of protected ribonucleotides currently known in the art, such as the organometallic route, are not 2'-3' selective for the introduction of the protecting group. This method allows the selective introduction of the methylthiomethyl group at the 2' position and thereby prevents an unselective step late in the synthesis scheme.

In this new route a 2'-O-alkythiomethyl-ribonucleoside as can be represented by the formula



wherein  $R_7$  is alkyl or aryl or a combination of alkyl and aryl, is reacted with a silanol of the general formula  $\text{HOSiR}_4\text{R}_5\text{R}_6$ . In a preferred embodiment  $R_7$  is a  $(\text{C}_1$  to  $\text{C}_{20})$  -, more preferred is  $(\text{C}_1$  to  $\text{C}_{10})$ - alkyl and/or aryl. In another preferred embodiment  $R_7$  is for instance methyl, ethyl, propyl, butyl, pentyl, hexyl, iso-propyl, sec-butyl, iso-butyl, sec-pentyl. The substituents  $R_4$ ,  $R_5$  and  $R_6$  of the silanol are identical or different alkyl or aryl or a combination of alkyl and aryl substituents, or substituted heteroatoms and which may also cyclically be connected to each other. In a preferred embodiment the three substituents together comprise between 3 and 30 carbon atoms each.

In another preferred embodiment, compound 11 is reacted with a silanol of the general formula  $\text{HOSiR}_4\text{R}_5\text{R}_6$  with the substituents  $R_4$ ,  $R_5$ ,  $R_6$  as defined above, under suitable conditions known in the art. These comprise an electrophilic activating reagent or reagent combination such as, but not limited to, for instance N-halosuccinimides and a catalytic amount of an acid. The reaction may be carried out in solution or on solid phase or by using polymer supported reagents. The solvent can be a hydrocarbon solvent, ethereal solvent, nitrile solvent, chlorinated solvent, heterocyclic solvent, sulfoxide solvents, etc.. Specific examples of suitable solvents include pyridine, N,N-dimethylformamide (DMF), tetrahydrofuran (THF), dimethylsulfoxide (DMSO), acetonitrile, dichloroethane and methylene chloride. Preferably, dichloromethane is used. Although the reaction may be carried out at room temperature, it may also be carried out at a temperature of  $-78^\circ\text{C}$  to  $100^\circ\text{C}$  preferably at 0 to  $50^\circ\text{C}$ .

The alkythiomethyl-substituent at the 2'-OH group comprises preferably a  $(\text{C}_1$  to  $\text{C}_{12})$ -alkyl and/or aryl group, preferably a  $(\text{C}_1$  to  $\text{C}_6)$ -alkyl and/or aryl group. Examples of preferred substituents comprise methylthiomethyl, ethylthiomethyl, propylthiomethyl, isopropylthiomethyl or butylthiomethyl.

The following Examples illustrate the present invention, without in any way limiting the scope thereof.

## EXAMPLES

### 1. Preparation of THEX building blocks via organometallic route

Scheme 1 depicts the synthetic scheme for the introduction of the THEX protecting group on 5'-O-DMTr Uridine and the subsequent phosphitylation.

#### Preparation of (1,1,2-trimethyl-propyl)-dimethylsilyloxymethyl chloride (THEX-Cl)

A suspension of 11.1ml (0.15mol) ethanethiol and 4.5g (0.15mol) para-formaldehyde was treated with two drops of NaOMe/MeOH (30%) and stirred 1h at 40°C. After cooling, 150ml CH<sub>2</sub>Cl<sub>2</sub> and 22.66g (0.333mol) imidazole were added. After 10 minutes, 32.66g (0.167mol) (1,1,2-trimethyl-propyl)-dimethylsilyl chloride was added dropwise. The resulting suspension was stirred at room temperature for 24 hours and diluted with 300ml n- hexane. After adding 200ml 2M NaH<sub>2</sub>PO<sub>4</sub> solution, stirring (15 minutes) and phase separation, the organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The residue was dissolved in 100ml CH<sub>2</sub>Cl<sub>2</sub>, treated dropwise with 12.3ml (20.4g, 0.152mol) sulfurylchloride in 50ml CH<sub>2</sub>Cl<sub>2</sub>. After 1 hour, the mixture was evaporated. The product was obtained (31.5g) as wax.

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): 0.5 (s, 6H, SiMe<sub>2</sub>); 0.65 (12H, CH<sub>3</sub>); 1.40 (sept, 1H, CH); 5.43 (s, 2H, CH<sub>2</sub>).

#### Preparation of 1-[5'-O-(4,4'-Dimethoxytrityl)-2'-O-(((1,1,2-trimethyl-propyl)-dimethylsilyl)]-oxymethyl-beta-D-ribofuranosyl]-uracil (5a)

A solution of 9.5g (17.4mmol) 5'-O-dimethoxytritylated uridine(1) in 200ml 1,2-dichloroethane was treated with 11.23g (87mmol) Huenig's base and then with 5.81g (19.2mmol) dibutyl-tindichloride. After 30 minutes, the mixture was heated to 80°C, treated with 4.2g (22.6mmol) (1,1,2-trimethyl-propyl)-dimethylsilyloxymethyl chloride (THEX-Cl) in 50ml dichloroethane and stirred two hours at 80°C. After cooling, the mixture was diluted with 400ml CH<sub>2</sub>Cl<sub>2</sub> and 350ml aqueous saturated NaHCO<sub>3</sub> solution were added. After stirring 30 minutes, the layers were separated and the organic layer was evaporated. The residue was chromatographed

on silica gel, using ethylacetate/hexane(3:1) containing 0.1% N-methylmorpholine. The product was obtained as solid foam (4.52g).

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): 0.1 (s,6H,CH<sub>3</sub>); 0.6-0.8 (s and d,12H,CH<sub>3</sub>); 1.45 (m,1H,CH); 3.15 (d,2H,CH<sub>2</sub>); 3.64 (s,6H,OCH<sub>3</sub>); 3.85 (q,2H,CH<sub>2</sub>); 4.05 (m,1H,CH); 4.15 (m,1H,CH); 4.80 (q,2H,CH<sub>2</sub>); 5.12 (d,1H,OH); 5.30 (q,1H,CH); 5.78 d,1H CH); 6.8-5.3 (m,13H); 7.6 (d,1H).

Preparation of 1-[5'-O-(4,4'-Dimethoxytrityl)-2'-O-(((1,1,2-trimethyl-propyl)-dimethylsilyl))-oxymethyl-beta-D-ribofuranosyl]-uracil-3'-(2-cyanoethyldiisopropyl)phosphoramidite(6)

A solution of 4.0g (5.56mmol) protected uridine(2), 1.14g (6.68mmol) diisopropylaminotetrazolid and 2.01g (6.68mmol) bis(N,N-diisopropylamino)-2-cyanoethoxy phosphine in 150ml CH<sub>2</sub>Cl<sub>2</sub> were stirred 24 hours at roomtemperatur. The mixture was diluted with 100ml CH<sub>2</sub>Cl<sub>2</sub> and washed twice with 50ml aqueous saturated NaHCO<sub>3</sub> solution. The dried (Na<sub>2</sub>SO<sub>4</sub>) organic phase was evaporated and the residue was subjected to column chromatography (ethylacetate/hexane 3:2 with additional 0.1% N-methylmorpholine). The product was obtained as a solid foam (4.12g).

<sup>31</sup>P-NMR (400 MHz, CDCl<sub>3</sub>): 151.183 (s) and 151.537 (s).

**2. Preparation of THEX building blocks via 2'-O-methylthiomethyl route**

3',5'-O-Diacetyl-2'-O-methylthiomethyl-uridine(8)

A solution of 4.53g (14.8mmol) 2'-O-methylthiomethyl uridine 7 (8) in 50ml pyridin was treated with 3.04g (29.7mmol) Ac<sub>2</sub>O. After 24h stirring, the solution was evaporated. The residue was dissolved in 40ml EtOAc, washed with water and dried with Na<sub>2</sub>SO<sub>4</sub>. After evaporation, the pure title-compound was obtained (5.05g).

3',5'-O-Diacetyl-2'-O-(((1,1,2-trimethyl-propyl)-dimethylsilyl))-oxymethyl]-uridine (9)

0.33g (1.47mmol) N-iodosuccinimid in 3 ml THF was added to a solution of 0.5g (1.287mmol) 8, 0.978g (6.1mmol) (1,1,2-trimethyl-propyl)-dimethyl silanol, 10ml CH<sub>2</sub>Cl<sub>2</sub> and 1 drop MeOSO<sub>3</sub>H. After 2h stirring, 2ml NaHSO<sub>3</sub> (37%) was added, then 100ml CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was separated and dried with Na<sub>2</sub>SO<sub>4</sub>. After filtration, the solution was evaporated: 552mg pure title-compound.

2'-O-(((1,1,2-trimethyl-propyl)-dimethylsilyl))-oxymethyl]-uridine(10)



A solution of 187mg (0.37mmol) 9, 20ml MeOH and 0.135 ml (0.74mmol) NaOMe/MeOH (30%) was stirred 30min. at 0°C. After evaporation, the residue was filtered through a small silica gel column (EtOAc/MeOH 4:1): 140mg 10 as powder.

**3. Procedure for the incorporation of 6 into oligodeoxynucleotide by phosphoramidite chemistry and fast deprotection thereof**

Oligonucleotide synthesis were typically performed on an ABI394 automated DNA synthesizer (Applied Biosystems). DNA Phosphoramidites, THEX protected Uridine phosphoramidite (6) or TOM protected Uridine phosphoramidite (Xeragon, Inc.) were dissolved in dry acetonitrile at a 5% w/v concentration; coupling was made by activation of phosphoramidites using a 0.2 M solution of benzimidazolium triflate (9) in acetonitrile. Coupling times were between 1-5 minutes. A first capping was made using standard capping reagents. Oxidation was made using an 0.1M iodine solution in THF/water/pyridine (1:1:1). A second capping was performed after oxidation. Detritylation before the next coupling was effected with 2% dichloroacetic acid in dichloroethane.

Upon completion of oligonucleotide chain elongation, the solid support was transferred to an Eppendorf tube.

When prepared with THEX protected Uridine phosphoramidite (6), oligonucleotides were cleaved from support and deprotected as follows:

1. 32% aq. Ammonia/EtOH 3:1 (250 µl for 0.2 µmole scale), room temperature, 2h lyophilisation to dryness.
2. 1M tetrabutylammonium fluoride in THF (250 µl for 0.2 µmole scale), 30 min at room temperature.
3. 1M Tris.HCl, pH=7.4 (250 µl for 0.2 µmole scale).

When prepared with TOM protected Uridine phosphoramidite, oligonucleotides were cleaved from support and deprotected as follows:

1. 32% aq. Ammonia/EtOH 3:1 (250 µl for 0.2 µmole scale), room temperature, 2h lyophilisation to dryness.

2. 1M tetrabutylammonium fluoride in THF (250  $\mu$ l for 0.2  $\mu$ mole scale), 6h min at room temperature.
3. 1M Tris.HCl, pH=7.4 (250  $\mu$ l for 0.2  $\mu$ mole scale).

Resulting crude solutions were analysed by Capillary Gel Electrophoresis.

Results are summarized in table 1

#	Sequence	TOM (purity%)	THEX (purity%)
<u>11</u>	TTT TTU TTT TTT TTT	85	79
<u>12</u>	TTT TTU UUU TTT TTT	67	72

As shown in table 1, and figures 1-4, quality of crude material obtained with THEX protected Uridine phosphoramidite 6 and TOM protected Uridine phosphoramidite are very similar. Use of 2'-O-THEX protecting group strategy allowed the reduction of 2' deprotection from 6h at 35°C (as reported in ref. 6) to 30 min at room temperature.

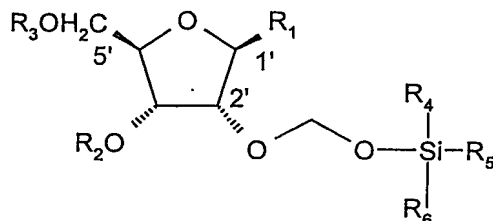
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## CLAIMS

1. A ribonucleoside-derivative of the formula



wherein

$R_1$  is a base of the purine- or pyrimidine-family or a derivative of such a base or any other residue which serves as a nucleobase surrogate,

$R_2$  is a proton or a substituted derivative of phosphonic acid,

$R_3$  is a proton or a protection-group for the oxygen atom in 5'-position,

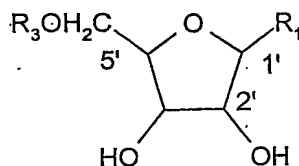
$R_4$ ,  $R_5$  and  $R_6$  are alkyl or aryl or a combination of alkyl and aryl or heteroatom,  $R_4$ ,  $R_5$  and  $R_6$  may also be cyclically connected to each other;

and

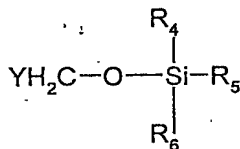
wherein at least one of the  $R_4$ ,  $R_5$  or  $R_6$  substituents comprises a tertiary C-atom or a heteroatom vicinal to the Si-atom.

2. A ribonucleoside-derivative according to claim 1 wherein the substituent comprising the tertiary C-atom vicinal to the Si-atom comprises from 4 to 24 C-atoms.
3. A ribonucleoside-derivative according to claim 1 wherein the substituent comprising the tertiary C-atom vicinal to the Si-atom is an alkyl-substituent selected from the group consisting of tert-butyl, tert-pentyl, tert-hexyl, tert-heptyl, tert-octyl, tert-nonyl, tert-decyl, tert-undecyl, tert-dodecyl.
4. A ribonucleoside-derivative according to claim 1 wherein the substituent comprising the tertiary C-atom vicinal to the Si-atom is selected from the group of 1,1-dimethyl ethyl, 1,1-dimethyl-propyl, 1,1-dimethyl-butyl, 1,1-dimethyl-pentyl, 1,1-dimethyl-hexyl, 1,1,2-trimethyl-propyl, 1,1,2-trimethyl-butyl, 1,1,2-trimethyl-pentyl, 1,1,2-trimethyl-hexyl, 1,1,2,2 tetramethyl-propyl, 1,1,2,2-tetramethyl-butyl.

5. A ribonucleoside-derivative according to claim 1 wherein the substituent vicinal to the Si-atom comprises a substituted heteroatom.
6. A ribonucleoside-derivative according to claim 4 wherein the substituent vicinal to the Si-atom comprises a substituted bivalent heteroatom.
7. A ribonucleoside-derivative according to claim 5 wherein the heteroatom is oxygen.
8. A method for the preparation of a ribonucleoside-derivative according to claim 1, comprising reacting a nucleoside with the formula



where  $R_1$  and  $R_3$  are as defined in claim 1, with a silyloxymethyl derivative of the formula

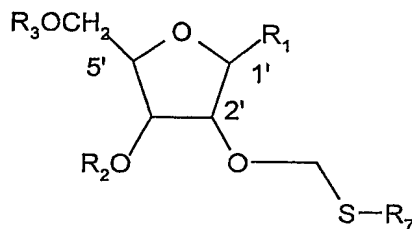


wherein Y is a suitable leaving group

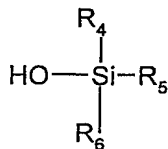
and wherein  $R_4$ ,  $R_5$  and  $R_6$  are alkyl or aryl or a combination of alkyl and aryl or a heteroatom,  $R_4$ ,  $R_5$  and  $R_6$  may also be cyclically connected to each other.

9. The method of claim 8 wherein Y is a halogen.
10. The method of claim 8 or 9 wherein  $R_4$ ,  $R_5$  and  $R_6$  together comprise between 3 and 30 carbon atoms.
11. The method of claims 8 or 9 wherein  $R_4$ ,  $R_5$  and  $R_6$  comprise at least one substituted heteroatom vicinal to Si atom.

12. The method of claim 8 or 9 wherein the heteroatom is a bivalent atom.
13. The method of claim 8 or 9 wherein the heteroatom is oxygen.
14. The method of claim 8 or 9 wherein the ribonucleoside derivative is further substituted on the oxygen in 3'-position with a group comprising of a derivative of phosphonic acid.
15. A method for the preparation of a ribonucleoside-derivative, comprising reacting a ribonucleoside derivative with the formula



upon an electrophilic activation with a compound of formula:

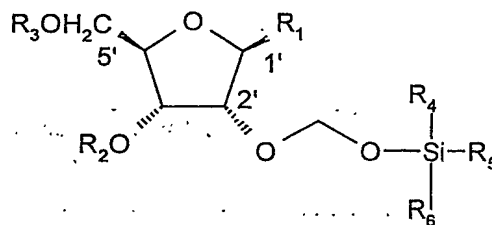


wherein  $R_1$  is defined as in claim 1 and  $R_7$  is a alkyl- or aryl-group, or alkyl-aryl-group,  
 wherein  $R_2$  is a protecting group,  
 wherein  $R_3$  is a protecting group,  
 wherein  $R_4$ ,  $R_5$  and  $R_6$  are identical or different alkyl or aryl or a combination of alkyl and aryl substituents, which may be further substituted with heteroatoms and which may also cyclically be connected to each other.

16. The method of claim 15 wherein  $R_4$ ,  $R_5$  and  $R_6$  are defined as in claims 1 to 7.
17. The method of claim 15 or 16 wherein the ribonucleoside derivative is further substituted on the oxygen in 3'-position with a group comprising of a derivative of phosphonic acid.

## ABSTRACT

The invention provides ribonucleoside derivatives with novel protecting groups and methods for the preparation of such ribonucleoside derivatives. The general formula of the ribonucleoside derivatives is:



wherein

- R<sub>1</sub> is a base of the purine- or pyrimidine-family or a derivative of such a base or any other residue which serves as a nucleobase surrogate,
- R<sub>2</sub> is a proton or a substituted derivative of phosphonic acid,
- R<sub>3</sub> is a proton or a protection-group for the oxygen atom in 5'-position,
- R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> are alkyl or aryl or a combination of alkyl and aryl or heteroatom, R<sub>4</sub>, R<sub>5</sub> and R<sub>6</sub> may also be cyclically connected to each other;
- and
- wherein at least one of the R<sub>4</sub>, R<sub>5</sub> or R<sub>6</sub> substituents comprises a tertiary C-atom or a heteroatom vicinal to the Si-atom.

PCT Application

**EP0313113**

